Spatiotemporal variation of bacterial water quality and the relationship with pasture land cover
Erin E. Scott, Mansoor D. K. Leh and Brian E. Haggard

ABSTRACT
Pathogens are a major cause of water quality impairment and public health concern world-wide. In the United States, each state is tasked with developing water quality standards (WQS) to protect the designated use(s) of waterbodies. Several streams in the Illinois River Watershed in northwest Arkansas are currently listed as impaired due to elevated levels of pathogens. Our objective was to evaluate *Escherichia coli* (*E. coli*) numbers at 29 stream sites, compare these numbers to the applicable WQS, and investigate the relationship between *E. coli* numbers and land cover variables. *E. coli* numbers in samples collected at most sites were within allowable limits, although there were several instances of violations of the WQS. Violations were variable from year to year at some sites, and elevated levels of *E. coli* were spatially localized during baseflow. Violations also were positively related to pasture land cover in the drainage area, and particularly within the riparian buffer area. This relationship was non-linear, or threshold based, where there was a significant increase in the mean *E. coli* exceedances when riparian pasture land cover was greater than approximately 50%.

These results can be used to identify specific stream reaches where *E. coli* numbers might be elevated and the implementation of best management practices can be geographically targeted.

Key words | *E. coli* bacteria, environmental regulations, geospatial analysis, riparian buffers, watershed management

INTRODUCTION
Pathogen contamination of water resources and subsequent human infection is a major water quality concern throughout the world, even in developed nations. In the United States, pathogens are listed as the most common cause of impairment resulting in waterbodies being added to the 303(d) list (United States Environmental Protection Agency [USEPA] 2016). The 303(d) list is a list developed by each state that identifies waterbodies that fail to meet their designated use(s) due to excess pollutants. Each state is tasked with developing water quality standards (WQS) for pathogens based on the amount of an indicator organism, such as *Escherichia coli* (*E. coli*), per unit volume of water, and applicable to the designated use(s) of a water body. Many streams and rivers are designated as primary or secondary contact waters, and the intent of the WQS is to protect human health during recreation.

Elevated numbers of *E. coli* in surface waters can result from a variety of sources (Arnone & Walling 2007) including runoff from adjacent land (Frenzel & Couvillion 2002; Ramos et al. 2006), leaking septic systems and sewage lines (Jamieson et al. 2004), and direct deposition by wildlife and grazing livestock (Bradford et al. 2013; Wilkes et al. 2015). Generally, the majority of bacteria loading to streams
occurs during rainfall runoff events from urban, agricultural, and even forested areas (Jamieson et al. 2003; Tyrrel & Quinton 2003; Krometis et al. 2007), which can cause significant increases in the number of indicator organisms above background levels (World Health Organization (WHO) 2003). Many studies have found that bacteria numbers increase with increasing discharge in streams (Christensen et al. 2002; Crowther et al. 2002), and the same was true for sites in the Upper Illinois River Watershed (UIRW) (David & Haggard 2011), the focus of our study. Bacteria such as E. coli can also survive for prolonged periods in stream bed sediments. For example, Garzio-Hadzick et al. (2010) found that E. coli survived for 30 to over 100 days in streambed sediments, and just 5 to 25 days in the water column. Bacteria can become resuspended during both storm-flow (Muirhead et al. 2004) and base-flow conditions (Sherer et al. 1988; Crabill et al. 1999; Jamieson et al. 2003), which can result in an immediate increase in bacteria in the water column (Garzio-Hadzick et al. 2010).

Agricultural activities on pasture land are often cited as a major source of bacteria pollution to streams. For example, pasture land can be used for cattle grazing and for land application of poultry litter as a fertilizer amendment, both of which can contribute to the transport of pathogens to adjacent waterways (Crowther et al. 2002; Weidhaas et al. 2011; Bradford et al. 2013). Additionally, cattle grazing activities within the riparian buffer area can decrease riparian vegetation and increase soil erosion (Agoridis et al. 2005; Grudzinski et al. 2016), influencing bacterial transport to streams. Many farmers and water resource managers have identified the need to implement best management practices (BMPs) to minimize the risk of bacterial transport into streams and rivers.

In the UIRW in northwest Arkansas, pasture land dominates the landscape (50%), where E. coli numbers in streams are likely influenced by livestock and agricultural activities on the landscape, wildlife, and/or by the resuspension of stream bed sediments. The specific objectives of this study were to: (1) evaluate baseflow E. coli numbers in streams on the 303(d) list for pathogens; (2) compare this data against the applicable WQS; and (3) investigate the relationships between E. coli numbers and land cover variables, particularly within the riparian buffer area. The goal of this paper is to allow regulators to make informed decisions on water-quality impairment and help water resource managers target areas to potentially improve water quality.

**MATERIALS AND METHODS**

This study focuses on the UIRW in Arkansas, United States, a transboundary watershed that originates in northwest Arkansas and flows into Oklahoma. The UIRW drains an area of 1,952 km², of which 50.3% is pasture and grassland, 35.9% is forest, 8.8% is urban and suburban, 4.3% is transitional, and 0.3% is water (arkansaswater.org 2015). The primary agricultural activities in the UIRW include cattle and poultry production. Land use throughout the watershed is also changing, with increases in residential, commercial, and industrial development.

Water samples were collected for E. coli analysis at 29 sites across 10 reaches in seven streams in the UIRW during base-flow conditions. All study reaches were on the 2008 303(d) list of impaired waterbodies for pathogens, with the source of impairment unknown (Arkansas Department of Environmental Quality (ADEQ) 2008). Water samples were collected eight or nine times during the primary contact season – May 1 through September 30 – during 2012, 2013 and 2014. Water samples were collected from the thalweg in sterile containers and transported on ice to the Arkansas Water Resources Center Water Quality Laboratory, certified for bacteria. E. coli numbers were analyzed using the IDEXX Colilert-24 Total Coliform and E. coli method (method 9223B; APHA 2005) and the most probable number of colonies/100 ml (MPN/100 ml) was reported.

Catchment areas and riparian zones were delineated using ArcGIS and ArcHydro tools (Environmental Systems Research Institute (ESRI) 2015), the 2011 United States Geological Survey (USGS) National Land Cover Dataset (Homer et al. 2015), and the National Hydrography Dataset. The riparian zones were delineated considering both the distance upstream from the sample location (0.5, 1, 2, 3, and 4 km upstream) and the width from the center of the stream channel (20, 30, and 45 m on each side). This resulted in a total of 15 (5 stream lengths × 3 buffer widths) extracted riparian zones upstream from each sample site. All tributaries that were within each distance...
upstream from the sample point were included in the delineation.

Bacterial numbers in the water samples were evaluated against the applicable WQS for Arkansas (Arkansas Pollution Control and Ecology Commission (APCEC) 2014). Specifically, the *E. coli* limit in all study streams is 410 MPN/100 ml, except for in the Illinois River where the limit is 298 MPN/100 ml due to its designation as an Ecologically Sensitive Waterbody. The regulation states that these limits for *E. coli* must not be exceeded in more than 25% of the samples in no less than eight samples collected during the primary contact season (May 1 through September 30). The percent of samples that exceeded the limit for *E. coli* was calculated for each sample site and year (‘site-year’). Geomean *E. coli* numbers were also calculated for site-years for use in linear regression and non-parametric change point analyses.

A simple linear regression and a non-parametric change point analysis (NCPA) (R Core Team 2016; King & Richardson 2005; Qian et al. 2003) were used to relate catchment and riparian land use land cover (LULC) to geomean *E. coli* numbers for site-years and to the percent of water samples that exceeded the limit for *E. coli*. NCPA is often used to analyze non-linear relationships between two environmental and/or biological variables. The NCPA analysis identifies a split in the data on the x-axis where there is a significant change in the mean and/or deviation around the mean between the two groups of data (data to the left and right of the split); this split is called the ‘change point’. NCPA also calculates uncertainty around the change point using bootstrapping and resamples the data with replacement to calculate the change point.

### RESULTS

*E. coli* numbers ranged from 1 to 11,780 MPN/100 ml across all the samples collected during the study period, where the greatest numbers occurred at a site on Little Osage Creek (LO933A; Table 1). While most sites never exceeded the applicable WQS, there were 11 instances of violations of the *E. coli* standard across site-years (Figure 1; Table 1).

One site on the Illinois River (IR028D) violated the WQS during each of the three years, where the *E. coli* limit was exceeded in 50–75% of water samples collected. All three sites on Little Osage Creek violated the WQS in 2012 and 2014, with 50–78% of water samples exceeding the limit for *E. coli*. One site on Baron Fork (BF013B) exceeded the *E. coli* limit in 38% of samples collected in 2012, and another site on the Illinois River (IR028A) violated the standard in 2014, with 33% of samples exceeding the limit for *E. coli* (Figure 1). Summary statistics for *E. coli* concentrations and percent exceedances at each site and for each year can be found in Table 1.

*E. coli* numbers increased linearly with increasing pasture in the drainage area ($r^2 = 0.13, p = 0.008$; Figure 2(a)). However, a non-linear change point or ‘threshold’ response explained more variability regarding this relationship (NCPA, $r^2 = 0.20, p = 0.002$; Figure 2(b)). Specifically, *E. coli* numbers increased significantly once the percentage of pasture in the drainage area exceeded 55%. The average *E. coli* numbers to the left and right of this change point were 73 and 201 MPN/100 ml, respectively (Figure 2(b)). Furthermore, high *E. coli* numbers occurred more frequently and with greater magnitude when pasture was greater than 55% compared to when pasture was less than 55%. For example, the maximum *E. coli* number was 271 MPN/100 ml to the left of the change point, and this value was exceeded nine times to the right of the change point, with a maximum of 958 MPN/100 ml.

Violations of the applicable WQS for *E. coli* were also influenced by the percentage of pasture land cover, particularly within the riparian buffer area of study streams. For example, for a defined riparian buffer area 3 km upstream from the sample site with a 30-m width, the change point occurred at 46% pasture land cover ($p = 0.003, r^2 = 0.22$; Figure 3). This means that when pasture was greater than 46% in the riparian buffer area, the average percent exceedance of the WQS was significantly greater than when pasture was less than 46%. In fact, the only sites that exceeded the WQS had greater than 46% pasture land cover in the riparian buffer area.

The amount of land area included in the riparian buffer zone affected the results of the change point analysis, where the percentage of pasture land cover that resulted in different average percent exceedances in the WQS varied as the definition for riparian buffer area varied. Figure 4 shows
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The table includes the number of samples collected (N), the geometric mean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) E. coli as the most probable number (MPN) of colonies/100 ml. The percentage of E. coli measurements exceeding the limit of 298 MPN/100 ml or 410 MPN/100 ml for the Illinois River sites and all other sites, respectively (% Exc.) is also shown. Bold values for % Exc. represent stream sites that violated the applicable WQS in a given year (E. coli numbers exceeded the limit for more than 25% of the samples collected; APCEC Regulation 2).

Figure 1 | Map showing exceedances across study sites in the Illinois River Watershed. The color of the site symbols represents the incidence of E. coli exceeding the standard of 298 MPN/100 ml in the Illinois River and 410 MPN/100 ml in all other rivers in more than 25% of the samples collected during the primary contact season (May 1 through September 30) of each year (APCEC Regulation 2). White, yellow, purple and dark blue symbols represent sites with 0, 1, 2 or 3 years of violations of E. coli standard. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2017.101.
that change points generally increased as the amount of area included in the riparian buffer delineation increased, from 0.5 to 4 km upstream from the sample location and as the width from the stream channel increased from 20 to 45 m on either side (all change points were significant at $\alpha = 0.05$). At the smallest defined areas, average percent exceedances across site-years increased significantly when pasture represented as little as 20% of the riparian area, whereas this change point occurred at 40–50% pasture in larger defined riparian areas. The confidence intervals about the change points were large in smaller riparian areas, and decreased as these buffer areas increased in size. Violations of the WQS only occurred when the percent pasture in the riparian buffer area was greater than the change point value (see Figure 3, for example). However, many site-years did not violate the WQS even when the percent pasture in the riparian buffer area was above the change point.

**DISCUSSION**

The intent of the WQS for *E. coli* for the primary contact season is to protect public health during body contact...
recreational activities, such as swimming. Users would typically recreate during base-flow conditions, after stormflow has receded. Therefore, we collected water samples during base-flow conditions and intentionally avoided storm events. It should be noted, however, that some users (e.g. white-water paddlers) may choose to recreate during elevated flows resulting from storm events. These users may be subjected to elevated levels of E. coli, regardless of watershed land use since bacterial numbers increase with increasing flow even in highly forested or pristine watersheds (Niemi & Niemi 1991).

In fact, past data for the Illinois River Watershed show that bacteria numbers during storm events increased dramatically relative to baseflow across streams draining agricultural to forested watersheds (Haggard, unpublished data; Figure 5). For example, sample sites with geomean E. coli numbers less than 100 MPN/100 ml during baseflow had elevated stormflow numbers that ranged from approximately 170 to 850 MPN/100 ml, often above the allowable limit. Similarly, sample sites with higher baseflow E. coli numbers, greater than 350 MPN/100 ml, had even higher stormflow geomean E. coli numbers that ranged from approximately 1,000 to 2,400 MPN/100 ml, well above the allowable limit. Thus, we sampled our study sites during base-flow conditions because we did not want to have the study sites inadvertently listed as violating the WQS because storm event data was included.

During summer base-flow conditions, the source of bacteria can be more localized and include direct deposition into the water by pets, wildlife, and livestock (Schumacher 2003; Wilkes et al. 2013). At low flow, bacteria are less likely to be transported great distances downstream due to slower water velocities, greater predation, the increased presence of pools, and increased settling into the stream bed sediments (Schumacher 2003; Bradford et al. 2013). Our results support the localized nature of elevated E. coli numbers during baseflow. For example, water samples exceeded the applicable E. coli limit in 15 out of 25 samples collected at one site on the Illinois River (IR028D), but only once out of 25 samples collected at a site just 7.7 km downstream (IR024A). In Arkansas, streams are divided into reaches and these entire reaches are listed as impaired (ADEQ 2008) even though elevated E. coli numbers might just occur at one site in a reach, and not further up or downstream along the same reach. Regulatory agencies might consider using this information to change the way in which streams are listed as impaired because often it is only segments, not entire reaches, which violate the WQS. Then, the efforts to remove the reaches from the 303(d) list could focus on areas near the site with elevated bacteria.

Our data also illustrate the importance of evaluating the applicable WQS over the course of multiple years due to the interannual variability of E. coli numbers at some sites. For example, both BF013B and IR028A violated the applicable WQS only in one of the three years that water samples were collected. There can be many reasons for differences in stream E. coli numbers across years including changes in the presence of cattle, temperature, flow, and nutrient availability. Many farmers implement rotational grazing of cattle as a BMP in order to reduce degradation of the landscape and water quality (Agouridis et al. 2005). Therefore, cattle may be present at a stream site at one sampling date or year, but not the next. Additionally, interannual variability in hydrology and weather can influence bacteria in the water column (Laurent & Mazumder 2014). In years when particularly favorable conditions exist, bacteria can persist for long periods of time in the sediments and later become resuspended upon disturbance, by wading cattle or recreational users for example (Sherer et al. 1988; Crabill et al. 1999). We recommend that multiple years of data be used to assess the WQS for bacteria in streams in order to
account for variability among years, and that some consideration be given to requiring a stream to exceed the standard more than one year within a defined period of time before classifying it as impaired.

The geomean *E. coli* numbers at stream sites in our study increased when pasture land cover increased in the catchment area. This increase in *E. coli* with pasture land is likely tied to multiple sources associated with agricultural runoff (Crowther *et al.* 2002; Bradford *et al.* 2015) and livestock activities within the catchment. For example, Crowther *et al.* (2002) showed that indicator organisms at several stream sites across two watersheds were strongly and positively related to the percentage of grazed grassland and/or land on which animal wastes were applied. In the Illinois River Watershed, both grazing by cattle and land-applied poultry litter as fertilizer amendments are common practices. While our study lacks data directly related to livestock activities and poultry litter application rates, we do show that once pasture land cover in the drainage area reached 55%, there was an increase in the average geomean *E. coli* numbers at sampling sites. Our analysis also suggests that more variance in *E. coli* numbers and WQS exceedances was explained by more localized land use, i.e. pasture land within the riparian zone.

Based on results from change point analyses that related the percent exceedance of the WQS for *E. coli* to riparian land use, we can identify specific stream reaches where *E. coli* numbers might be a problem during base-flow conditions and the implementation of BMPs can be geographically targeted. We recommend defining the riparian buffer area as 3 km upstream from the sampling site and 50 m wide from each side of the stream channel. Land use land cover data using ArcGIS was evaluated based on 30 m pixels, so using a buffer width of 30 m on each side of the stream channel (60 m total width) should adequately identify true land cover within the buffer area. Additionally, this definition for the riparian buffer area resulted in among the smallest range in confidence intervals, suggesting it can reliably be used to indicate where problem areas might occur and water sampling for indicator organisms might be appropriate. The World Health Organization (2005) suggests that appropriate authorities develop a program to evaluate existing hazards and monitor for changes in the area in order to address safety in recreational waters. Using GIS to target potential hot spot areas could be an important part of a monitoring program, since water sample collection and analysis at all possible sites could be cost prohibitive.

When we evaluated exceedances of the WQS using the recommended buffer dimensions, 31% of the site-year data exceeded the WQS for *E. coli* at sites where riparian land cover was greater than 47% pasture. However, 69% of the site-year data did not exceed the WQS, even when riparian land cover was greater than 47% pasture. What was different between the sites that violated the WQS and sites that did not? Direct cattle access to the stream channel can be an important factor driving high *E. coli* numbers in streams (Sherer *et al.* 1988; Schumacher 2003; Davies-Colley *et al.* 2004). One limitation of our study is that we lacked comprehensive data on cattle presence and stocking densities at and upstream from sampling sites. However, based on *post hoc* observations of cattle activity at each site (we visually surveyed each sample location at the end of the project), at five out of the six sites where violations of the WQS occurred, cows were seen on the landscape and were able to access the stream directly (e.g. no fencing was present to exclude cattle). Conversely, at half of the sites that had greater than 47% pasture land cover in the recommended buffer area but did not violate the WQS, we observed fencing near the stream channel and cattle on the landscape.

A variety of BMPs can be implemented to protect stream water quality from cattle activities on pasture lands. For example, Wilkes *et al.* (2015) demonstrated the success of cattle exclusion practices, where microbial source tracking markers for cattle were significantly lower at a stream site where cattle were excluded by fencing compared to a site where cattle had direct access to the stream channel. Providing shade sources and watering tanks away from the stream channel and riparian areas can also be effective in decreasing the impacts of cattle to stream water quality (Agouridis *et al.* 2005; Grudzinski *et al.* 2016). Finally, even when there is a high percentage of pasture land in a catchment, the presence of an intact forested riparian area can filter out pathogens and other contaminants and have positive impacts on stream water quality (Barling & Moore 1994; Zhang *et al.* 2010). Results from our study can be used to inform land owners and resource managers about potential problem areas for *E. coli* based on the amount of pasture...
land cover in the riparian area, and help guide the implementation of BMPs to improve water quality and reduce the risk to public health.

CONCLUSIONS

Most sites had *E. coli* numbers in collected water samples that were within allowable WQS limits during the three-year study. When *E. coli* levels in water samples were elevated, violations of the WQS were variable from year to year and were spatially localized. Regulatory agencies should consider the need to collect multiple years of bacteria or *E. coli* data to evaluate water quality impairment.

Additionally, pasture land cover in the riparian buffer area was positively related to exceedances of the WQS for *E. coli*, where exceedances only occurred when pasture cover was greater than 47%. Potential problem areas can be identified by evaluating the amount of pasture land in the riparian buffer area, and a water quality monitoring plan or BMPs can be targeted to these areas.

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